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ASSOCIATION OF CYTOCHROME P450 GENE POLYMORPHISM WITH LUNG CARCINOMA AMONG ADULT MALE SMOKER

Shilpa Jain,[a] Anil Goel,[b] Khushal Shah,[c]

[a] Professor, Department of Biochemistry, Medical College, Baroda & SSG Hospital, Vadodara.
[b] Professor & Head, Department of Radiation Oncology, Medical College, Baroda & SSG Hospital, Vadodara.
[c] Consultant Medical Biochemistry, Unipath Specialty Laboratory Limited, Ahmedabad.

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Corresponding author:

Shilpa Jain
Professor & Head,
Department of Biochemistry
Medical College Baroda &
SSG Hospital Vadodara
A-2/115, San Tropez,
Madrid County
Bhayali Road, Vadodara-
391410. Gujarat

Email
jinshilpa73@gmail.com

Abstract:

Background & Objective Lung Cancer is a major health problem worldwide with high fatality rate. Smoking is a known to be the most important risk factor, though all smokers do not develop lung cancer. This is because individuals differ in metabolism of pro-carcinogens present in cigarette smoke by cytochrome P450 (CYP 450) enzymes. Variable forms of CYP450 enzymes are produced due to presence of multiple alleles or polymorphs of CYP 450 gene. CYP 1A1 polymorphisms were the first CYP genes to be associated with lung cancer. Several single nucleotide polymorphisms (SNPs) have been identified in CYP1A1 gene, some of which are suspected to be linked with lung cancer. This study has been done to find association of CYP1A1 genes- m1, m2, and m4 polymorphism with lung cancer in heavy smokers. **Methods & Results** This is an observational analytical study on heavy smoker eighty males, of which forty were with lung cancer (Group I) and remaining forty were with non-lung cancer (Group II). CYP1A1 gene m1, m2 and m4 polymorphism was detected by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) techniques. Steps involved in these techniques are DNA isolation, polymerase chain reaction, DNA electrophoresis and restriction fragment length polymorphism. CYP1A1 m1 Gene polymorphism was found in 3 out of 22 (13.6%) cases of squamous cell lung cancer and only 1 case (4.5%) had both the m1 and m2 gene polymorphisms. None of these polymorphisms was seen in non-lung cancer smokers and in patients with non-squamous cell lung cancer. **Interpretation & Conclusion** CYP1A1 gene related m1 and m2 polymorphisms may have some contributory role for lung squamous cell carcinoma development and m4 polymorphism has no role in development of lung cancer in Indian population of eastern part of Gujarat. All lung carcinoma cases may not have gene polymorphism of CYP1A1 gene.

INTRODUCTION

Lung cancer is the most common cancer in males and fourth most common cancer in females worldwide.[1,2] In 2020, approximately 2 million new cases of lung cancer were diagnosed out of which 1.7 million deaths occurred, which is the most common cause of cancer related deaths worldwide.[2]

Smoking is the strongest established risk factor for lung cancer but it has been seen that not all individuals who are exposed to predisposing factors get carcinoma.[3,4] An individual's susceptibility to develop cancer depends on one's genetic make-up as well as environmental factors.[3,4]

Genetically determined variations in metabolism of tobacco derived carcinogens may affect an individual's susceptibility to lung cancer.[3,4] Fewer than 20% of smokers develop lung cancer, indicating that there may be important genetic components involved in the etiology.[3,4]

Tobacco smoke contains various procarcinogens like nitrosamines, aromatic amines, polycyclic aromatic hydrocarbons (PAH) including benzopyrenes (BP).[5] Xenobiotics like PAHs are metabolized in two phases.[5] CYP450 monooxygenase system is involved in phase one reactions in which Benzopyrene is converted to Benzopyrene-7,8-dihydrodiol-9,10-epoxide which can form guanine adducts on DNA and lead to mutations. These PAH derived carcinogens also interact with p53 gene and bind to aryl hydrocarbon receptor (AhR) and induce these Cyto P450 forms, thus increasing their own metabolism.[6,7]

Fifty-seven CYP450 isoforms of this CYP450 super family are known to be present in humans. These are microsomal membrane bound hemoproteins widely distributed among various forms of life and perform diverse physiological functions. These enzymes are encoded by CYP genes which show multiple allele forms (Polymorphism).[8] Polymorphism is normal variation of nucleotide sequence present in more than 1% of population and generally has no deleterious effect.[9]

There are 18 mammalian CYP450 families which encode 57 genes in human genome. These are named as CYP1, CYP2, CYP3 and so on.[10] Of these, CYP1A1 gene (Family 1, subfamily A, member 1) is first to be associated with lung carcinoma and is mainly expressed in extrahepatic tissues, including lung, where it is markedly induced by PAHs and its metabolites. It codes for CYP1A1 enzymes (EC 1.14.14.1) of CYP 450 superfamily which is involved in phase I xenobiotic metabolism.[6]

Elevated activity of Cytochrome P450 superfamily enzyme is associated with formation of more reactive intermediates that can form PAH-related DNA adduct leading to high lung cancer risk. Various polymorphisms found in lung cancer are; m1, m2, m3 and m4 polymorphism of CYP1A1 and these highly inducible forms of CYP1A1 are associated with an increased risk of lung cancer in smokers.[2-4,6]

This study has been designed to find out association of lung cancer with common gene polymorphisms of CYP1A1 gene.

MATERIAL & METHODS

This is an observational analytical case control study carried out from May 2016 to November 2016 in Biochemistry department, Medical College Baroda in collaboration with Radiation Oncology department of SSG Hospital & Medical College Baroda, Vadodara. Institutional Scientific Review Committee & Ethical Committee approval was taken.

Informed consent was obtained from all the subjects prior to enrolment in the study. Eighty heavy smoker (30 or more pack years smoking history) males were included in the study and grouped as follows;

Group I comprised of 40 male smokers with histo-pathologically proven primary lung cancer attending SSG Hospital Vadodara and Group II consisted of 40 male smokers with age & sex matched to group I patients attending SSG Hospital with diseases other than lung cancer.

Female patients, patients with other co-existing non respiratory origin carcinoma, lung

secondaries and smokers who left smoking for more than 15 years were excluded from the study.

Informed consent and detailed medical history of the patients including personal data, present complaints, treatment history, past history, family history and personal history were taken. Standard general and loco-regional examination was carried out.

In addition to the routine biochemical and pathological investigations advised by clinician, 5 ml venous blood samples were also collected in EDTA vacutainer for gene polymorphism analysis, and were stored at -20°C temperature till the analysis for gene polymorphism was done.

Gene polymorphisms were detected by PCR-RFLP techniques. Steps involved in these techniques were DNA isolation, polymerase chain reaction, DNA electrophoresis and restriction fragment length polymorphism.[11-13]

Following gene polymorphisms were looked for-

(1) m1: T→C substitution at nucleotide 3801, in the 3'-non-coding region.

(2) m2: A→G substitution at nucleotide 2455, leading to an amino acid change of isoleucine to valine at codon 462.

(3) m4: C→A substitution at nucleotide 2453, leading to an amino acid change of threonine to asparagine at codon 461.

Reagents

Forward primers and reverse primers for all these polymorphic alleles were obtained from Sigma Aldrich. MAGTaq 5X Blood Direct PCR Master Mix Kit was obtained from APS LABS. (Catlog number- MAGSPIN-93) and MAGBand 100 bp DNA Ladder was obtained from APS LABS (Catlog number- MAGSPIN-21).[13]

Restriction enzyme Msp1 for m1 polymorphism was obtained from THERMO SCIENTIFIC (Catlog number- ERO541), BsrD1 for m2 polymorphism was obtained from PUREGENE (Catlog number- PGR261) and Bsa 1 for m4 polymorphism was obtained from THERMO SCIENTIFIC (Catlog number- ERO291).[13]

Polymorphism Analysis

PCR reaction mixtures were prepared in biosafety cabinet by adding PCR master mix, forward & reverse primers, distilled water and whole blood sample from EDTA vacutainer for all the three types of polymorphisms. These were then amplified on PCR machine. This was followed by gel electrophoresis on 1.8% Agarose gel containing ethidum bromide against the DNA ladder on horizontal DNA gel electrophoresis. Presence of DNA was confirmed by visualizing gel under UV rays. After confirming the presence of DNA in the PCR product, presence of gene polymorphism was detected by treating with corresponding restriction enzymes followed by gel electrophoresis and visualization under UV illumination.[12] Gain of a restriction site occurs in the polymorphic allele; the wild type allele shows a single band and variant allele results in two DNA bands.[12]

Statistical Tests

Differences in the distribution of demographic and genotypic characteristics of cases and controls were evaluated using the MedCalc statistical software. We applied student t test for continuous variables and the frequency of gene polymorphism of both the groups have been depicted in percentage.

RESULTS & DISCUSSION

The patients in two group were age and sex matched with mean age of 60.30 years in Group I versus 60.43 years in Group II (p value= 0.93) and all patients were males. All patients in both the groups were heavy smokers with smoking history of more than 30 pack years (35.85 pack years in Group I versus 36.70 pack years in Group II, p value= 0.41).

Table I shows case distribution of patients in Group I among various types of lung carcinoma and Table II shows case distribution of patients in Group II among non-cancer patients.

Table I. Case distribution of patients in Group-I among various types of lung carcinoma

Type of lung carcinoma	No. of Patients (40)	Percentage
Small cell lung cancer	07	17.5%
Squamous cell lung cancer	22	55.0%
Adenocarcinoma	08	20.0%
Large cell carcinoma	03	7.5%

Table II. Case distribution of patients in Group-II among various non-lung cancer diseases

Disease	No. of Patients (40)	Percentage
Pulmonary Tuberculosis	13	32.5%
Emphysema	12	30.0%
Chronic Asthma	9	22.5%
Interstitial lung disease	6	15.0%

Table III and IV show, m1 & m2 polymorphism frequency, in CYP1A1 gene, among group-I and group-II patients, respectively.

Table III. m1- polymorphism distribution CYP1A1 gene in Group-I and Group-II

m1 Polymorphism	Group I (Lung Cancer)	Group II (Non-cancer)	Total
Polymorphism present	3 (7.5%)	0	3 (3.75%)
Polymorphism absent	37 (92.5%)	40 (100%)	77 (96.25%)
Total	40	40	80

Table IV. m2 polymorphism distribution CYP1A1 gene in Group-I and Group-II

M2 Polymorphism	Group I (Lung Cancer)	Group II (Non-cancer)	Total
Polymorphism present	1 (2.5%)	0	1 (2.5%)
Polymorphism absent	39 (97.5%)	40 (100%)	39 (97.5%)
Total	40	40	80

Figure I, shows two separate bands of DNA in well no. 19 & 20 showing presence of m1 polymorphism on gel electrophoresis. In well no. 3, 16, 17 & 25 there were failed attempts for DNA band detection. These samples were repeated. Well no. 1, 2, 4 to 15, 18, 21 to 24

showed single band of DNA. These samples were negative for detection of gene polymorphism in the DNA.

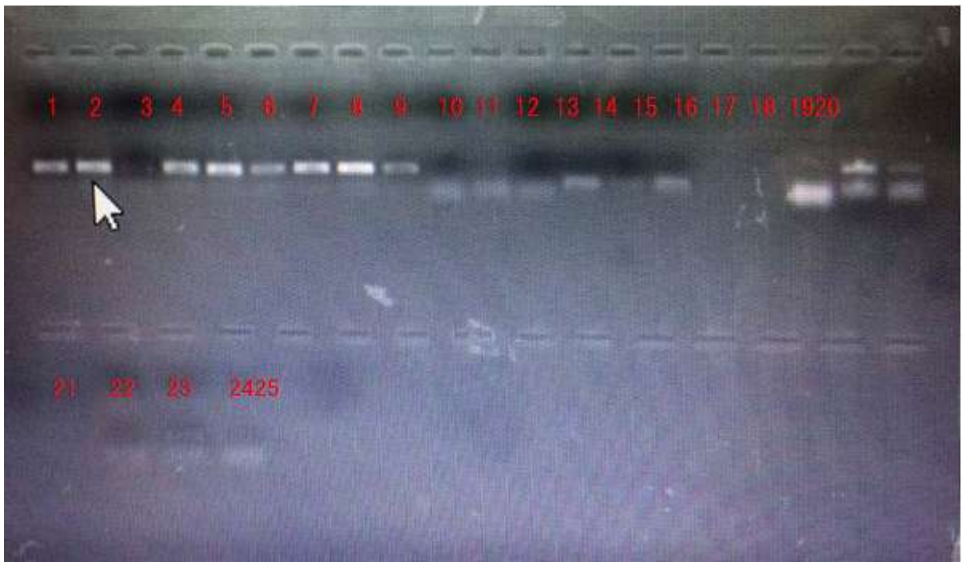

Figure I: Gel electrophoresis showing two separate bands of DNA due to gene polymorphism in well no. 19 & 20.

In our study m1 polymorphism in CYP1A1 gene was seen in 3 cases (7.5%) of lung carcinoma (Group-I), and not in any of the non-carcinoma patients (Group-II). The m2 polymorphism in CYP1A1 gene was seen in only one case (2.5%) of lung carcinoma patients (Group-1), and not in any of the non-carcinoma patients (Group-II). The m4 polymorphism was not detected in any of the cases in either group.

Table V. Cases showing CYP1A1 gene polymorphism

Sr. No.	Sample No.	Age (years)	Smoking (pack years)	Type of lung cancer	Stage of lung cancer	Polymorphism detected
1	14	60	31	Squamous cell	Stage III	m1 and m2
2	35	67	34	Squamous cell	Stage III	m1
3	36	54	42	Squamous cell	Stage II	m1

In our study gene polymorphism/s were found positive in squamous cell lung carcinoma cases only. Gene polymorphism was not found positive in any of small cell, large cell or adenocarcinoma. As shown in Table V, Gene polymorphism was seen in 3 out of 22 (13.6%) cases of squamous cell lung cancer. Out of these squamous cell lung cancer cases (22), two cases (9.1%) had single m1 gene polymorphism and only one case (4.5%) had both the m1 and m2 gene polymorphisms.

In our study, we found that majority of lung carcinoma patients were of squamous cell lung carcinoma (55%) followed by adenocarcinoma (20%), small cell carcinoma (17.5%) and large cell lung carcinoma (7.5%). Literature review reveals similar results. Shaffi et al have reported 63.3% of lung cancer cases as squamous cell carcinoma, 16.5% of adenocarcinoma and 20.2% of other types[4]. Study by Jose et al also shows almost similar results[14].

In our study, we found that 37.5% of lung carcinoma cases were of stage -III disease, which was the most common stage of presentation in our study, and percentage of stage-I, stage-II and stage-IV were 10%, 35% and 17.5% respectively.

In this study we looked for m1, m2 and m4 polymorphisms of CYP1A1 gene. Table VI shows comparison of m1, m2 and m4 polymorphisms between lung cancer (Group-1) and non-cancer (Group-2) in our study and different studies reported in literature.

Table VI. m1, m2 and m4 polymorphisms among different studies in group-1 and group-2

Studies	**Group-1 (%)**			**Group-2 (%)**		
	polymorphism			Polymorphism		
	m1	m2	m4	m1	m2	m4
Our study	7.5	2.5	0	0	0	0
Sheikh M et al[4]	74.3	67.9	0	52.1	48.5	0
Sanjose et al[14]	9.7	1.0	8.7	16.6	0.4	7.6
Hung R et al[15]	2.3	3	ND	2.3	1.5	ND
Cantlay et al[5]	16	ND	ND	12	ND	ND
Girdhar et al[16]	57	34	ND	47.6	19	ND

It was found that most common polymorphism of CYP1A1 gene among lung cancer patients as m1 type, followed by m2 type and no detection of m4 type.

It was also found that all lung carcinoma patients do not have genetic polymorphism present in their genetic structure, but only 7.5% of all lung carcinoma patients or 13.5% of squamous cell cancer were detected positive for genetic polymorphism. None of the non-carcinomatous patients (group-2) were detected positive for genetic polymorphism.

Shaffi et al found stronger association of CYP1A1 polymorphisms for development of lung carcinoma.[4] They observed that CYP1A1m1 and CYP1A1m2 variants were significantly associated with lung cancer susceptibility (ORs; 2.65, CI 95% = 1.562-4.49 and 2.24, CI 95%=1.35-3.73).[4] Hung et al found this odds ratio was 2.99 [95% confidence interval (95%CI) 1.51±5.91].[15]

In a study by Girdhar et al, there was a significant difference in the frequency distribution of the mutant m1 genotype of CYP1A1 between cases and controls and this study clearly demonstrated a significant association between the m1 genotype and lung cancer risk, and it was more pronounced in squamous cell carcinoma[16].

This difference in our findings compared to other studies could be due to difference in subject selection. In our study, we selected all heavy smokers with more than 30 pack years smoking history with mean± SD (range) for smoking pack year was 35.85 ± 4.17 (31- 44) for group-1 and 36.70 ± 4.93 (31-48) for group-2. In Shaffi et al study, only 77% lung cancer patients were smokers and out of which only 33% were smokers with more than 25 pack years of smoking history.[2] In Jose et al study, mean ± SD for cases was 61.2 ± 28.5, while for control it was 29.9 ± 21.4 with significant

difference ($p< 0.001$) between cases and controls for smoking history.[3]

Cigarette smokers have a 10-fold or greater increased risk of developing lung cancer compared to those who have never smoked. A deep sequencing study suggested that one genetic mutation is induced for every 15 cigarettes smoked.[8,17]

People who have smoking exposure of more than 30 pack years are on increased risk of lung cancer development and who are smoking more than 25 cigarettes per day have increased risk of death due to lung cancer (relative risk 23.7).[9] In our study to check the effect of gene polymorphism only, we chose heavy smokers in both the groups.

Our findings suggest that gene polymorphisms may have contributory role for development of lung carcinoma but it is not necessary that all lung carcinoma patients should have gene polymorphism as its etiological factor, other factors may also have major role. Detection of gene polymorphism can help in prediction of a person's susceptibility to develop lung carcinoma and provide them early diagnosis and treatment. Only the persons, who have positive family history of lung carcinoma, may get benefit from gene polymorphic detection. Their genetic makeup can give prediction, whether they have higher risk of developing lung carcinoma in future with their smoking habits.

Strength of this study was that both the groups were matched with respect to age, sex and duration & extent of smoking, thereby minimizing the confounding factors.

Limitations of this study are- small sample size, self-funding, high cost per test for genetic polymorphism detection. Positive polymorphism results are limited in Group I and none in Group II showed presence of polymorphism, so results have been presented as descriptive data. To establish strong association between gene polymorphism and lung cancer, study needs to be further carried on as case control study with more number of patients, considering other risk factors like industrial smoke, domestic smoke, vehicle smoke etc. in addition to cigarette smoke with statistical analysis

CONCLUSION

Squamous cell lung carcinoma is more common among heavy smokers and patients with this type of lung cancer express gene polymorphism more frequently compared to other types of lung carcinoma.

The m1 and m2 gene polymorphisms of CYP1A1 gene may have some contributory role for lung carcinoma development and m4 polymorphism has no role for lung carcinoma development in Indian population of eastern part of Gujarat.

All lung carcinoma cases may not have gene polymorphism of CYP1A1 gene suggesting that, other environmental risk factors may have rather major contribution for etio-pathogenesis of lung carcinoma.

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